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Oral flora of domestic cats in Hong Kong: Identification of antibiotic-resistant strains

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Abstract

Background: As the clinical outcome of bite-associated infection is related to the oral commensals, evaluating their composition and antibiotic susceptibility pattern can provide more information for the antibiotic treatment of wound infections and increase the awareness of the multidrug-resistant bacteria in cat oral flora.

Aims: This study was conducted to identify the various bacterial species in the oral cavity of cats. It aimed to identify the composition of cat oral flora and antibiotic resistant bacterial stains.

Materials and Methods: Twenty-two cats were sampled for bacterial evaluation. Matrix-assisted laser desorption/ionization time of flight mass spectrometry was used to provide rapid and reliable detection and identification of the bacterial species. Antibiotic susceptibility tests were performed in the identified isolates to determine the antibiotic susceptibility pattern and to detect the multidrug-resistant bacteria in the cat oral cavities.

Results: A total of 54 isolates were identified, *Pasteurella* was the genus most commonly isolated from the oral cavity of cats (19/54, 35.19%), followed by *Neisseria spp.* (8/54, 14.81%) and *Staphylococcus spp.* (7/54, 12.96%). Uncommon oral flora were isolated from the samples, including *Pasteurella canis*, *Inquilinus limosus* and the *Enterobacteriaceae* family of *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens*. Fourteen different multidrug-resistant bacteria were detected, including *Pasteurella* species (4/14), *Bacillus* species (2/14), *Neisseria* species (3/14), *Escherichia* species (1/14) and *Staphylococcus* species (4/14).

Discussion and Conclusions: This study's findings will increase the understanding of the composition of cat oral flora in Hong Kong, which can provide more evidence-based information for the prophylactic treatment of patients with cat bite infections. Moreover, the study identified and detected the antibiotic resistance pattern and multidrug-resistant bacteria in the cat oral cavity, which can help cat owners increase their awareness of maintaining regular oral hygiene for their cats to prevent the spread of pathogens from cats to humans.

Ka Tik Cheung and Hau Yan Chan contributed equally to this work.

KEYWORDS
antibiotic susceptibility test, cat oral flora, domestic house cats, *Inquilinus limosus*, multidrug-resistant bacteria, zoonotic diseases

1 | INTRODUCTION

Cats are one of the most popular pets in Hong Kong. According to the thematic household survey done by the Census and Statistics Department, a total of 167,600 cats were kept as pets by households in 2011. However, cat owners or animal care workers may be easily injured by a cat bite and develop a bite-associated infection. Worldwide, dog and cat bites are the most common bites injuries (Abrahamian & Goldstein, 2011). Among these bite-wound cases, the most common causes of morbidity are local skin and soft tissue infections; sepsis, fractures, osteomyelitis, meningitis and endocarditis can also be seen in high-risk patients, such as children or the elderly as they are immunocompromised (Smith et al., 2000). In addition to bite-associated diseases, many other mechanisms exist for transmitting zoonotic pathogens from cats to humans, such as kissing, hand-to-mouth transfer of microorganisms, cysts or oocysts and aerosolization of respiratory secretion, resulting in the development of infection (Abrahamian & Goldstein, 2011). Therefore, pet owners need to be aware of the human acquisition of zoonotic diseases from domestic house cats.

Distinct habitats in the oral cavity, such as the mucosal surface of the lips, cheeks, palate and tongue, can help support the growth of a distinctive microbial community. The mean pH of 6.75–7.25 of saliva in the mouth also favours the growth of many microorganisms, resulting in resident oral microflora formation (Scannapieco, 1994). Although oral flora is relatively harmless to the host, they can become significant pathogens, producing local or systemic disease once they leave the local site (Schuster, 1999), which occurs in bite wounds. A deep puncture wound is often a concern in feline bites, as the sharp and long teeth of cats can easily penetrate human skin creating a deep wound with a small opening with microorganisms inoculated into the subcutaneous soft tissues or even deeper into the periosteum (Abrahamian & Goldstein, 2011). As the bacteria recovered from bite wounds are reflective of the oral flora of the biting animal, only in a minority of cases do the pathogenic bacteria come from the victim's skin or the physical environment at the time of injury (Abrahamian & Goldstein, 2011). Thus, identification and evaluation of the oral flora of cats can aid in disease treatment. Studies have shown that the causative bacteria of animal bites are usually associated with *Pasteurella* species, *Staphylococcus aureus*, *Capnocytophaga canimorsus*, *Streptococcus species* and anaerobic bacteria (Dendle & Looke, 2008). Of these, the *Pasteurella* species are the predominant isolate from cat bite wounds, responsible for 75% of all infections (Talan et al., 1999).

Many research studies have identified the common cat oral flora or pathogens found in the infections caused by cat bite wounds. However, no previous research has mentioned the cat oral flora in a Hong Kong population. Different environmental sources or types of cat food may contain different types of bacteria (Ducey et al., 2016), and the number of biofilms can be affected by conditional variants, such as temperature, pH, redox potential, atmospheric conditions and salinity (Marsh & Zaura, 2017). Therefore, the cat oral flora from Hong Kong may be different in comparison to other countries, and identification of bacteria is necessary to treat cat bite infections in Hong Kong.

Although most oral flora are commensal species, they can become pathogenic in response to changes in the environment or other triggers in the oral cavity, including the quality of human personal hygiene (Avila et al., 2009). Bite wounds are usually a polymicrobial infection that consists of common environmental flora in addition to infectious agents specific to the saliva of the biting animals because bacteria in the oral cavity can associate to form biofilms, which can be resistant to mechanical stress or antibiotic treatment. Management of these wounds in the emergency department generally consists of wound washout, debridement and tetanus immunization (Hurt & Maday, 2018). However, wounds from a cat bite are often difficult to debride and disinfect due to the small opening of the injured area, so empiric prophylactic antibiotics are recommended for all cat bite cases because early and appropriate treatment can prevent infective complications of bite wounds (Hurt & Maday, 2018). It has been reported that 30%–50% of cat bite cases seen in emergency departments become infected if antibiotic prophylaxis is not prescribed to the patient (Rothe et al., 2015). At this stage, a board-spectrum of antibiotics is recommended to treat animal bites as the wounds tend to be polymicrobial in nature; however, there is an increased risk that the bacteria will be resistant to these drugs. Hence, it is necessary to evaluate the oral flora of domestic cats in Hong Kong and to determine their antibiotic resistance characteristics in order to provide an effective prophylactic treatment for patients who suffer from infections due to cat-bite wounds.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Samples were collected from the cats in veterinary clinics during their regular check-up or scheduled vaccinations. Twenty-two cats were selected; all were clinically healthy and had no history of surgery, anaesthesia or antimicrobial exposure 2 weeks before the sample collection period. Two specimens were collected from each animal using a sterile cotton-tipped applicator. Swabs were taken from the oral cavity by rotating the cotton tip on the floor of the oral cavity between the larynx and the mandibular teeth. After swabbing, the swab was transported in an Amies transporting media (Deltalab, Rubí, Barcelona, Spain) and inoculated in a blood agar medium (Oxoid, Thermo Fisher Scientific, USA) and a chocolate agar medium (Oxoid, Thermo Fisher Scientific, USA) within 2 h.

2.2 | Bacterial identification

The oral swab was inoculated and cultured in a chocolate agar medium and two blood agar mediums using the streaking method. One of the swabs cultured in the blood agar medium and the swab cultured in the chocolate agar medium were incubated for 18–24 h at 37°C under a rich CO_2 environment. The other swab cultured in the blood agar medium was placed in the anaerobic condition for 48 h. After incubation, the morphology and size of the colonies, including pigmentation, were examined (Mouro et al., 2010). Different isolates were differentiated by their varying morphology and subcultured into a corresponding agar. The culture plates were further incubated overnight to obtain a single colony for bacteria identification. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry was used to provide rapid and reliable detection and identification of the bacterial species. This automated platform can provide an effective identification of bacteria at the species level. The test was performed by placing a bacterial colony on a disposable target slide using a wooden stick and adding 1-μl direct formic acid to cover it. After the formic acid overlay was completely dried, 1-μl light standard matrix solution was added directly to the slide, and then the slide was. Based on the protein profile, identification of the microorganism is performed by comparing the spectra to the database of reference spectra (Dingle & Butler-Wu, 2013).

2.3 | Antibiotic susceptibility test

The antibiotic susceptibility test on each isolate was performed using the disk diffusion method to determine the antibiotic susceptibility pattern and to identify the multidrug-resistant bacteria from the cat oral flora. The antibiotic disks were selected among those frequently used in Hong Kong and available for clinical practice (Mouro et al., 2010). According to the Clinical and Laboratory Standards Institute (CLSI) (*M100 Performance Standards for Antimicrobial Susceptibility Testing. A CLSI Supplement for Global Application. 28th Edition*, n.d.), a standardized bacterial inoculum was applied to the entire surface of a Mueller–Hinton agar plate (Oxoid, Thermo Fisher Scientific, USA) or a blood Mueller–Hinton agar plate (Oxoid, Thermo Fisher Scientific, USA) prior to the sterile application of thin paper disks impregnated with the antimicrobial agents to be tested. The plates were incubated at 35°C under varied conditions for an appropriate duration based on the CLSI guidelines, and the zone of inhibition was examined to measure the diameter of the area in which the growth of the organism was inhibited by the antibiotic. The results were interpreted using the zone size ranges with individual antimicrobial agents, established by CLSI, to determine whether the organisms are sensitive, intermediate or resistant to the antibiotics.

2.4 | Detection of extended-spectrum beta-lactamase (ESBL)

Extended-spectrum beta-lactamase (ESBL) is commonly present in Enterobacteriaceae. Therefore, it is essential to detect ESBL as it has the potential to transfer to other organisms via conjugation from the plasmid gene. CLSI recommends routine testing of *Escherichia coli*, *Klebsiella* spp., and *Proteus mirabilis* for the presence of ESBL. According to CLSI guidelines, ceftazidime and cefotaxime alone and in combination with clavulanic acid were used in the disk diffusion method, as clavulanic acid is a kind of beta-lactamase inhibitor and beta-lactamase of the organisms is susceptible to it. An increase greater than 5 mm of the zone of inhibition of the antibiotic plus clavulanic acid in the disk indicates an organism that produces ESBL.

2.5 | Statistical method

Descriptive statistics are used to describe the presence and the frequency of different types of bacteria with graphics analysis.

2.6 | Ethics statement

All the cats were recruited from animals brought in by their owners to veterinary clinics in Hong Kong for routine health checks and vaccinations. No invasive procedures were required for the sample collection. Participation was voluntary. Informed client consent was obtained with approval from the Human and Animal Subject Ethics Subcommittee at Tung Wah College.

3 | RESULTS

3.1 | Bacterial identification

Demographic information of the samples is shown in Table 1, including age, sex, breed, number of isolates and frequency of oral hygiene. The frequency of oral hygiene is calculated by the number of times the cats underwent dental cleaning procedures from a veterinarian from the date of the cat's birth to the day of the sample collection. A total of 144 bacteria were isolated from the 22 cat samples. Among the 144 bacteria, 80 (55.6%) were aerobes, 55 (38.2%) were facultative anaerobes and 9 (6.2%) were obligate anaerobes (Table 2). Moreover, 35% (50/144) were Gram-positive bacteria and 65% (94/144) were Gram-negative bacteria. Thus, the oral cavities of the 22 cats contained more Gram-negative bacteria than Gram-positive bacteria.

TABLE 1 Demographic information of the samples

Sample ID	Age	Sex	Breed	Number of isolates
1	4	M	DSH	5
2	2	M	DSH	5
3	2	F	DSH	3
4	4	F	DSH	3
5	10	F	DSH	6
6	2.5	M	American Shorthair	9
7	1.5	F	Exotic Shorthair	6
8	10	M	Cocaine cat	7
9	7	M	Mix with British Shorthair	6
10	13	M	DSH	10
11	18	M	DSH	5
12	6	M	Munchkin	6
13	3	M	Chinchilla	4
14	12	M	DSH	7
15	5	F	British Shorthair	6
16	11	M	DSH	3
17	6	M	DSH	5
18	20	M	DSH	11
19	17	F	DSH	11
20	7	M	DSH	8
21	6	M	Munchkin	13
22	11	M	Chinchilla	5
	Range: 1.5–20 mean: 8.1	M: 16 F: 6		Range: 3–14 mean: 6.5

Note: The age, sex, breed and the number of isolates per sample were shown. The mean age of the cats is 8.1. There are 16 male feline and 6 female feline samples.

TABLE 2 Total bacterial isolates ($n = 144$) from 22 samples

Type of bacteria ($n = 144$)	Frequency	Total isolation (%)
Aerobe	80	55.6
Facultative anaerobe	55	38.2
Obligate anaerobe	9	6.2

Note: Among the 144 bacteria, 80 (55.6%) were aerobe, 55 (38.2%) were facultative anaerobes and 9 (6.2%) were obligate anaerobes. Overall, 35% (50/144) were Gram-positive bacteria and 65% (94/144) were Gram-negative bacteria. It showed that there were more Gram-negative bacteria identified in the cat's oral cavity.

TABLE 3 Isolates obtained from the oral cavity of cat

Species of bacteria isolated from the oral cavity of cats		
Organisms	**Frequency**	**Total isolation (%)**
Gram-positive bacteria		
Staphylococcus spp.	7	12.96
Micrococcus luteus	3	5.56
Bacillus cereus group	2	3.7
Streptococcus ovis	1	1.85
Corynebacterium aurimucosum	1	1.85
Actinomyces meyeri	1	1.85
Total	15	27.78
Gram-negative bacteria		
Pasteurella spp.	19	35.19
Neisseria spp.	8	14.81
Pseudomonas spp.	3	5.56
Escherichia coli	2	3.7
Fusobacterium nucleatum	2	3.7
Klebsiella pneumoniae	1	1.85
Serratia marcescens	1	1.85
Inquilinus limosus	1	1.85
Acinetobacter baumannii complex	1	1.85
Bacteroides pyogenes	1	1.85
Total	39	72.22
Grand total	54	100

Note: The bacteria identified from the 22 samples were shown in the table. Among the total isolated 114 bacteria, a total of 54 isolates were identified with 16 different genera. The most frequently isolated Gram-positive bacteria were *Staphylococcus* spp. (7/54, 12.96%), followed by *Micrococcus* spp. (3/54, 5.56%) and *B. cereus* group (2/54, 3.7%). However, the most frequently isolated Gram-negative bacteria were *Pasteurella* spp. (19/54, 35.19%), followed by *Neisseria* spp. (8/54, 14.81%), *Pseudomonas* spp. (3/54, 5.56%), *E. coli* (2/54, 3.7%) and *F. nucleatum* (2/54, 3.7%).

Among the 144 isolates, 74.31% (107/144) were catalase-positive organisms and 25.69% (37/144) were catalase-negative organisms. Moreover, 29.16% (42/144) of the organisms had a positive oxidase reaction and 70.83% (102/144) had a negative oxidase reaction (102/144). For the coagulase test, 33.33% (48/144) of the organisms were coagulase-positive and 66.66% (96/144) were coagulase-negative. The bacteria identified from the 22 samples are shown in Table 3. Among the 114 isolated bacteria, 54 isolates were identified with 16 different genera. The most frequently isolated Gram-positive bacteria were *Staphylococcus* spp. (7/54, 12.96%), followed by *Micrococcus* spp. (3/54, 5.56%) and the *Bacillus cereus* group (2/54, 3.7%). The most frequently isolated Gram-negative bacteria were *Pasteurella* spp. (19/54, 35.19%), followed by *Neisseria* spp. (8/54, 14.81%), *Pseudomonas* spp. (3/54, 5.56%), *E. coli* (2/54, 3.7%) and *Fusobacterium nucleatum* (2/54, 3.7%).

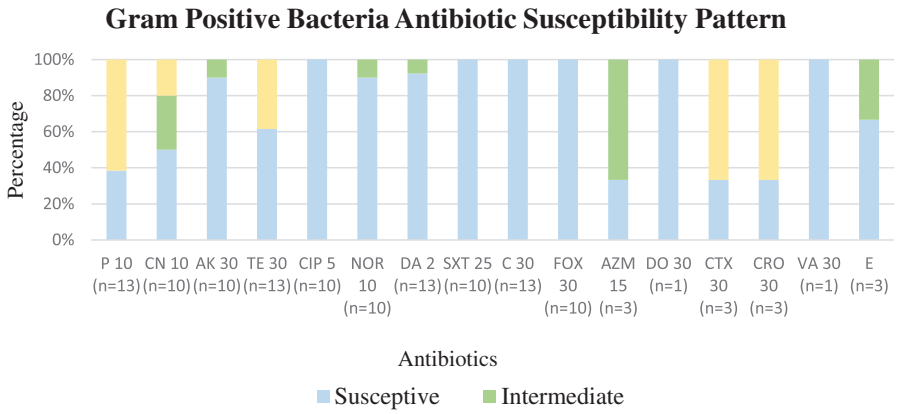

FIGURE 1 Antibiotic susceptibility pattern in Gram-positive bacteria all isolates were fully sensitive to trimethoprim/sulfamethoxazole, chloramphenicol, cefoxitin, doxycycline and vancomycin (100%). However, patterns of 66.67% resistance to cefotaxime and ceftriaxone and 61.54% resistance to penicillin were being observed.

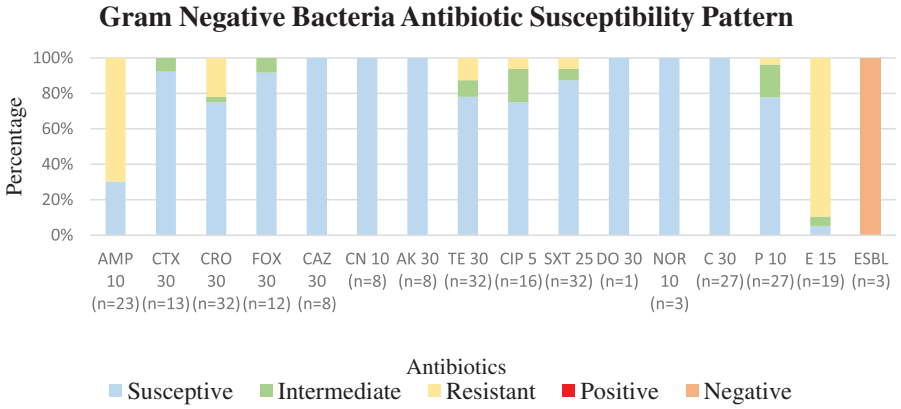

FIGURE 2 Antibiotic susceptibility pattern in Gram-negative bacteria: All isolates were fully sensitive to ceftazidime, gentamicin, amikacin, doxycycline, norfloxacin and chloramphenicol (100%). However, negative results were shown in the extended-spectrum beta-lactamase (ESBL) detection in all the three isolates. Patterns of 69.57% resistance to ampicillin and only 5.26% susceptible to erythromycin were being observed.

3.2 | Antibiotic susceptibility test results

Antibiotic susceptibility testing (AST) was performed in 48 isolates based on the CLSI guidelines, of which 27.1% were Gram-positive organisms. As shown in Figure 1, all the tested isolates were 100% sensitive to trimethoprim/sulfamethoxazole, chloramphenicol, cefoxitin, doxycycline and vancomycin. A pattern of 66.67% resistance to cefotaxime and ceftriaxone and 61.54% resistance to penicillin was observed. For the Gram-negative organisms, all the isolates were 100% sensitive to ceftazidime, gentamicin, amikacin, doxycycline, norfloxacin and chloramphenicol. ESBL was detected in *E. coli* and *Klebsiella* spp. A pattern of 69.57% resistance to ampicillin and 5.26% susceptibility to erythromycin was observed (Figure 2).

3.3 | Antibiotic susceptibility test on an individual genus

Fourteen different multidrug-resistant bacteria were found in this study, including *Pasteurella* species (4/14), *Bacillus* species (2/14), *Neisseria* species (3/14), *Escherichia* species (1/14) and *Staphylococcus* species (4/14). Each of the multidrug-resistant bacteria showed non-susceptibility (intermediate or resistant) to at least one antibiotic in three or more antimicrobial categories. In this study, the multidrug-resistant *Pasteurella* species were commonly resistant to ampicillin, ceftriaxone and erythromycin, which belong to the penicillin, cephalosporin and macrolide categories, respectively. The isolated strains showed 100% susceptibility to penicillin, chloramphenicol and trimethoprim/sulfamethoxazole. A pattern of 89.5% resistance to erythromycin was observed (Figure 3a). For the multidrug-resistant *Neisseria* species, the isolated strains showed 100% susceptibility to cefotaxime and chloramphenicol, but no unique antimicrobial susceptibility pattern was observed (Figure 3b). The isolates were commonly resistant or intermediate to penicillin, tetracycline and ciprofloxacin, which belong to the penicillin, tetracycline and fluoroquinolone categories, respectively. However, the isolates were commonly susceptible to cefotaxime and chloramphenicol. All the *Staphylococcus* isolates showed 100% susceptibility to ciprofloxacin, norfloxacin, clindamycin, trimethoprim/sulfamethoxazole, chloramphenicol and cefoxitin. A pattern of 71.43% resistance to penicillin and tetracycline and 28.57%

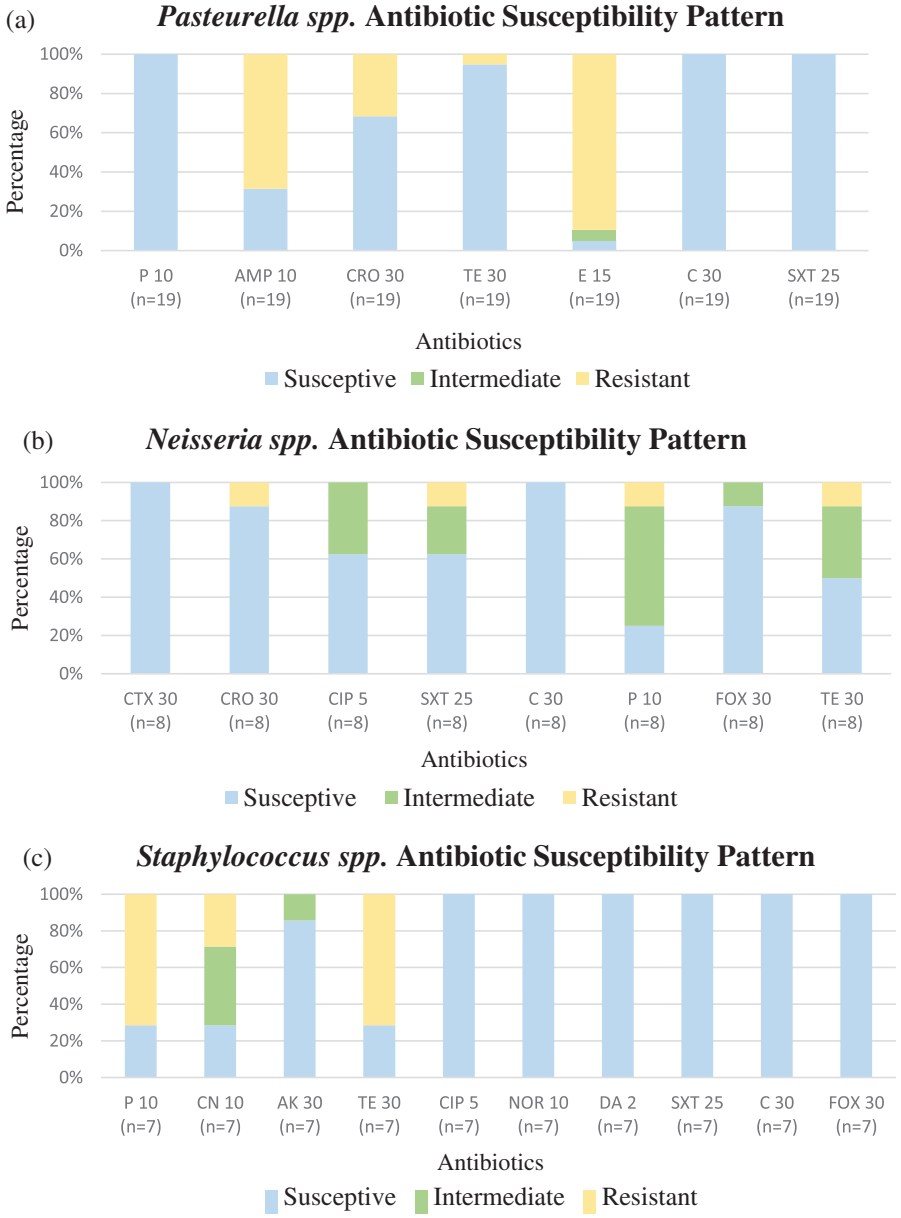

FIGURE 3 Antibiotic susceptibility pattern in (a) *Pasteurella* spp., (b) *Neisseria* spp. and (c) *Staphylococcus* spp. (a) The isolated strains showed 100% susceptible to penicillin, chloramphenicol and trimethoprim/sulfamethoxazole. A pattern of 89.5% resistance to erythromycin was being observed. (b) The isolated strains showed 100% susceptible in cefotaxime and chloramphenicol, no unique antimicrobial susceptibility pattern was being observed. (c) The isolated strains showed 100% susceptible to ciprofloxacin, norfloxacin, clindamycin, trimethoprim/sulfamethoxazole, chloramphenicol and cefoxitin. Patterns of 71.43% resistance to penicillin and tetracycline and only 28.57% susceptible to gentamicin were being observed.

susceptible to gentamicin was observed (Figure 3c). All of the isolates also showed non-susceptibility to at least one agent in the penicillin, tetracycline and aminoglycosides categories. They were susceptible to agents in the cephalosporin, phenicol, folate pathway inhibitor, lincosamides and fluoroquinolone antibiotic categories. For the multidrug-resistant *Bacillus* species, both strains showed non-susceptibility to penicillin, ceftriaxone, cefotaxime and azithromycin, which belong to the penicillin, cephalosporin and macrolide categories, respectively. Only two of the isolates were also susceptible to tetracycline and chloramphenicol. A multidrug-resistant *E. coli* was identified in this study, which showed resistance to ampicillin, tetracycline, trimethoprim/sulfamethoxazole and ciprofloxacin in four antimicrobial categories, including penicillin, tetracycline, folate pathway inhibitor and fluoroquinolone. However, the *E. coli* isolate showed susceptibility to all the agents in the cephalosporin and aminoglycoside categories. Four multidrug-resistant *Staphylococcus* species were isolated; they showed non-susceptibility to at least one agent in the penicillin, tetracycline and aminoglycoside categories. They were susceptible to agents in the cephalosporin, phenicol, folate pathway inhibitor, lincosamide and fluoroquinolone antibiotic categories. The

multidrug-resistant patterns of the different isolates are summarised in Table 4.

4 | DISCUSSION

In this study, the predominance of these isolated strains in cat oral flora were also observed in other studies in relation to cat bite wound infections, such as *Pasteurella* spp. and *Neisseria* spp. (Talan et al., 1999). Therefore, the present study proved that the bacteria found in cat bite infections are associated with the oral flora of cats. Among the isolated bacteria obtained from the feline oral cavities, the Enterobacteriaceae family, including *E. coli*, *Klebsiella pneumoniae* and *Serratia marcescens*, *Pasteurella canis* and *Inquilinus limosus* were the uncommon oral flora isolates. *E. coli*, *K. pneumoniae* and *S. marcescens* are normal intestinal flora in animals and they can be isolated from a variety of environmental sources (Guentzel, 1996). The presence of uncommon oral flora isolates can be explained by the habit of self-grooming in cats. Sometimes cats lick their genital areas or anal regions after urination and elimination, as a clean-up procedure, especially when the stool is sticky or watery. Thus, cats can acquire intestinal bacteria in their mouth when they lick their anal regions and intestinal flora. The bacteria from faecal material can be transmitted to the mouth of cats and thereby exist as oral flora.

I. limosus was identified in one of the samples. It is a novel pathogen first isolated from the oral cavities of cats, and it was found in a lung transplanted cystic fibrosis (CF) patient in 2002 (Coenye et al., 2002). They were exclusively found in the sputum samples from CF patients (Chiron et al., 2005; Cicatiello et al., 2014; Wellinghausen et al., 2005). However, one isolate of *I. limosus* has been recovered from a blood sample of a prosthetic valve endocarditis patient, without CF, and it was reported as the sole microorganism related to the disease (Kiratisin et al., 2006). The pathogenic role of the *Inquilinus* species remains to be characterized as it was not a typical pathogen. Therefore, the pathogenic potential, source and epidemiology remain unknown, and further studies are needed to evaluate the clinical implications of and optimal therapeutic management for *I. limosus*. Studies have also reported that *I. limosus* is a new intrinsically multidrug-resistant species and usually shows a multi-resistant profile to antimicrobial drugs, including penicillin, cephalosporins, kanamycin, tobramycin, fosfomycin, colistin and doxycycline (Chiron et al., 2005; Cicatiello et al., 2014). However, in the present study, the antibiotic susceptibility pattern of this isolate could not be determined because no standard guideline could be obtained. Thus, the test standard should be modified to obtain the antimicrobial susceptibility pattern of this strain. For example, the minimum inhibitory concentration (MIC) method can be used instead of the disk diffusion method when performing the test.

The choice of drugs used in the AST was based on CLSI recommendations and the frequency of usage for clinical practice in Hong Kong. Among the 54 identified isolates, 40 individual isolates could process the AST. It was found that all the tested isolates were fully susceptible to chloramphenicol. Chloramphenicol is an excellent antimicrobial agent that has a broad spectrum of activity against most Gram-positive and Gram-negative bacteria, and clinically important anaerobic bacterial species, including *Fusobacterium*, *Clostridium* spp. and *Bacteroides fragilis* (Heal et al., 2009). The drug can inhibit protein synthesis by reversibly binding to the 50S subunit of the bacterial 70S ribosome, therefore preventing the attachment of the aminoacyl-tRNA to its binding region. However, chloramphenicol cannot be used as the drug of the first choice for bite infections due to its toxicity profile. It can only be used to treat severe infections due to susceptible pathogens when other less toxic regimens are ineffective or contraindicated (Hooton, 1999). Haematologic toxicity is the most critical side effect associated with chloramphenicol; the direct inhibition of mitochondrial protein synthesis can lead to bone marrow suppression of all cell lines (Schroeter, 1974). Therefore, chloramphenicol is not suitable for prophylactic treatment even though it has broad-spectrum activity against many clinically important bacteria.

The oral commensal of the cats can be transmitted to humans, and a zoonotic infection from cats to humans can occur. Humans and domestic house cats may share a close relationship and the zoonotic pathogens can be transmitted through different mechanisms, including kissing, biting, hand-to-mouth transfer of microorganisms and aerosolization of respiratory secretion (Abrahamian & Goldstein, 2011). Therefore, people should be aware of the human acquisition of zoonotic diseases from domestic house cats. As various bacteria are found in the feline oral cavity, some isolated species, including *Pasteurella* species, *K. pneumoniae* and *S. marcescens*, can be transmitted to humans and result in disease. Cat ownership by immunocompromised people may carry risk and they may be prone to having an infection when the cat's oral flora is transmitted to them accidentally. These opportunistic pathogens may also spread to and be transmitted by direct contact between cats and humans.

In the present study, the multidrug-resistant *Pasteurella* species were commonly resistant to ampicillin, ceftriaxone and erythromycin. Thus, it is recommended that these drugs not be used to treat an infection caused by *Pasteurella* species. For the multidrug-resistant *Bacillus* species, the interpretation was followed with the *Streptococcus* spp. β-haemolytic group in CLSI. As shown in Table 4, the strains showed non-susceptibility to penicillin, ceftriaxone, cefotaxime and azithromycin. The antimicrobial resistance pattern results in this study are in accordance with the findings of other research studies in which multidrug-resistant *Bacillus cereus* was isolated in fresh vegetables, raw cow milk and processed milk (Min Park et al., 2018). The multidrug-resistant *Bacillus* species were isolated in the present study; therefore, the public should be aware of the emergence of *Bacillus* species resistance in cat oral flora. A single multidrug-resistant *E. coli* was identified in this study, which showed resistance in four antimicrobial categories, including penicillin, tetracycline, folate pathway inhibitors and fluoroquinolones. Four multidrug-resistant *Staphylococcus* species, all of them were non-susceptible to at least one agent in the penicillin, tetracycline and aminoglycoside categories. In summary, there are quite numerous antimicrobial resistance bacteria found in oral flora of the cat. Therefore, cat owners should also increase their awareness of the transmission of multidrug-resistant species from cats to humans to prevent multidrug-resistant infection.

TABLE 4 Multidrug-resistant pattern on individual bacteria

Antibiotic	Interpretation	Antibiotic	Interpretation	Antibiotic	Interpretation
Penicillins		**Penicillins**		**Penicillins**	
P 10	S	P 10	S	P 10	S
AMP 10	R	AMP 10	R	AMP 10	R
Cephalosporins		**Cephalosporins**		**Cephalosporins**	
CRO	R	CRO 30	R	CRO 30	R
Tetracycline		**Tetracycline**		**Tetracycline**	
TE 30	R	TE 30	S	TE 30	S
Macrolides		**Macrolides**		**Macrolides**	
E 15	R	E15	R	E15	R
Phenicols		**Phenicols**		**Phenicols**	
C 30	S	C 30	S	C 30	S
Folate pathway inhibitors		**Folate pathway inhibitors**		**Folate pathway inhibitors**	
SXT 25	S	SXT 25	S	SXT 25	S
Total non-susceptibility	4/6 categories		3/6 categories		3/6 categories
Pasteurella multocida		***Bacillus cereus group***		***Bacillus cereus group***	
Antibiotic	**Interpretation**	**Antibiotic**	**Interpretation**	**Antibiotic**	**Interpretation**
Penicillins		**Penicillins**		**Penicillins**	
P 10	S	P 10	R	P 10	R
AMP 10	R	**Cephalosporins**		**Cephalosporins**	
Cephalosporins		CRO 30	R	CRO 30	R
CRO 30	R	CTX 30	R	CTX 30	R
Tetracycline		**Tetracycline**		**Tetracycline**	
TE 30	S	TE 30	S	TE 30	S
Macrolides		**Macrolides**		**Macrolides**	
E15	R	E 15	I	E 15	S
Phenicols		AZM 15	I	AZM 15	I
C 30	S	**Phenicols**		**Phenicols**	
Folate pathway inhibitors		C 30	S	C 30	S
SXT 25	S	**Lincosamides**		**Lincosamides**	
		DA 2	I	DA 2	S
Total non-susceptibility	3/6 categories		4/6 categories		3/6 categories
Neisseria zoodegmatis		***N. zoodegmatis***		***Neisseria weaveri/zoodegmatis***	
Antibiotic	**Interpretation**	**Antibiotic**	**Interpretation**	**Antibiotic**	**Interpretation**
Penicillins		**Penicillins**		**Penicillins**	
P 10	R	P 10	I	P 10	I
Cephalosporins		**Cephalosporins**		**Cephalosporins**	
FOX 30	I	FOX 30	S	FOX 30	S
CTX 30	S	CTX 30	S	CTX 30	S
CRO 30	R	CRO 30	S	CRO 30	S
Tetracycline		**Tetracycline**		**Tetracycline**	
TE 30	R	TE 30	I	TE 30	I
Phenicols		**Phenicols**		**Phenicols**	
C 30	S	C 30	S	C 30	S

(Continues)

TABLE 4 (Continued)

Neisseria zoodegmatis		*N. zoodegmatis*		*Neisseria weaveri/zoodegmatis*	
Antibiotic	**Interpretation**	**Antibiotic**	**Interpretation**	**Antibiotic**	**Interpretation**
Folate pathway inhibitors		**Folate pathway inhibitors**		**Folate pathway inhibitors**	
SXT 25	S	SXT 25	I	SXT 25	I
Fluoroquinolones		**Fluoroquinolones**		**Fluoroquinolones**	
CIP 5	I	CIP 5	I	CIP 5	I
Total non-susceptibility	4/6 categories		4/6 categories		4/6 categories
Escherichia coli		*Staphylococcus intermedius*		*S. intermedius*	
Antibiotic	**Interpretation**	**Antibiotic**	**Interpretation**	**Antibiotic**	**Interpretation**
Penicillins		**Penicillins**		**Penicillins**	
AMP 10	R	P 10	R	P 10	R
Cephalosporins		**Cephalosporins**		**Cephalosporins**	
CTX 30	S	FOX 30	S	FOX 30	S
CRO 30	S	**Tetracycline**		**Tetracycline**	
FOX 30	S	TE 30	R	TE 30	R
CAZ 30	S	**Phenicols**		**Phenicols**	
Tetracycline		C 30	S	C 30	S
TE 30	R	**Folate Pathway Inhibitors**		**Folate Pathway Inhibitors**	
Folate Pathway Inhibitors	SXT 25	S	SXT 25	S	
SXT 25	R	**Lincosamides**		**Lincosamides**	
Fluoroquinolones		DA 2	S	DA 2	S
CIP 5	R	**Fluoroquinolones**	**Fluoroquinolones**		
Aminoglycosides	CIP 5	S	CIP 5	S	
CN 10	S	NOR 10	S	NOR 10	S
AK 30	S	**Aminoglycosides**	**Aminoglycosides**		
		CN 10	I	CN 10	R
		AK 30	S	AK 30	I
Total non-susceptibility	4/6 categories		3/8 categories		3/8 categories

Staphylococcus intermedius		*S. intermedius*	
Antibiotic	**Interpretation**	**Antibiotic**	**Interpretation**
Penicillins		**Penicillins**	
P 10	R	P 10	R
Cephalosporins	**Cephalosporins**		
FOX 30	S	FOX 30	S
Tetracycline		**Tetracycline**	
TE 30	R	TE 30	R
Phenicols		**Phenicols**	
C 30	S	C 30	S
Folate Pathway Inhibitors		**Folate Pathway Inhibitors**	
SXT 25	S	SXT 25	S
Lincosamides		**Lincosamides**	
DA 2	S	DA 2	S

(Continues)

TABLE 4 (Continued)

Staphylococcus intermedius		*S. intermedius*	
Antibiotic	**Interpretation**	**Antibiotic**	**Interpretation**
Fluoroquinolones		**Fluoroquinolones**	
CIP 5	S	CIP 5	S
NOR 10	S	NOR 10	S
Aminoglycosides		**Aminoglycosides**	
CN 10	I	CN 10	R
AK 30	S	AK 30	S
Total non-susceptibility	3/8 categories		3/8 categories

Note: A total of 14 different individual multidrug-resistant bacteria were found in this study, including *Pasteurella* species, *Bacillus* species, *Neisseria* species, *Escherichia coli* and *Staphylococcus* species. Each of the multidrug–resistant bacteria was showing non-susceptibility (intermediate or resistant) to at least one antibiotic in three or more antimicrobial categories.
Abbreviations: I, intermediate; R, resistant; S, susceptible.

As the choice of antibiotics used in this study was limited, 18 common antimicrobial agents were chosen for Gram-positive and Gram-negative bacteria to perform the AST. Multidrug-resistant bacteria were observed by showing their non-susceptibility to at least one agent in three or more antimicrobial categories in the panel. However, some of the categories only contained one agent for the test, such as fluoroquinolones and lincosamides. Therefore, more antibiotics should be investigated to obtain a more accurate result for further study. Moreover, the MIC method should be used on the isolates if the disk diffusion method is not recommended by CLSI. A more precise and reliable result can then be obtained if MIC is used. Furthermore, this study only evaluated 22, so the sample size was too low to derive the statistical significance, as the frequency of isolates was not fully representative of the population. Therefore, the sample size should be increased in a future study.

5 | CONCLUSIONS

Pasteurella spp., *Neisseria* spp. and *Staphylococcus* spp. were the genera most commonly isolated from the oral cavity of cats in this study. Penicillin can be used when *Pasteurella multocida* is found as the sole pathogen in a cat bite infection. However, the drug should not be used alone in empiric antibiotic therapy because *Staphylococcus* spp. showed resistance to it. As multidrug-resistant bacteria were detected in this study, it is suggested that humans should be aware that they can acquire zoonotic diseases from domestic house cats. Moreover, future studies should investigate the clinical impact of *I. limosus* on the unusual isolates that were identified in the oral cavities of the cats. It is recommended that immunocompromised individuals that own cats should increase their awareness of cat oral commensal because they carry higher risks and are more prone to having a severe infection than non-immunocompromised individuals when cat oral flora is transmitted to them accidentally.

AUTHOR CONTRIBUTIONS

Conceptualization; investigation; methodology; supervision; writing – review and editing: Ka Tik Cheung. *Data curation; formal analysis; methodology; writing – original draft*: Hau Yan Chan.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

All the cats were recruited from animals brought in by their owners to veterinary clinics in Hong Kong for routine health checks and vaccinations. No invasive procedures were required for the sample collection. Participation was voluntary. Informed client consent was obtained with approval from the Human and Animal Subject Ethics Subcommittee at Tung Wah College.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supplementary Information of this article.

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PEER REVIEW

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